REMARKS

A Petition for Extension of Time is being concurrently filed with this Amendment. Thus, this Amendment is timely filed.

Applicant respectfully requests the Examiner to reconsider the present application in view of the foregoing amendments to the claims and the following remarks.

Status of the Claims

Claims 1-16 are currently pending in the present application. The Office Action is non-final. Claim 1-3 and 5-6 have been amended without prejudice or disclaimer. No new matter has been added by way of the amendments, because the amendments are supported by the present specification and they further define and clarify the structure of the present invention. In addition, support for claim 1 can be found within the present specification at page 5, lines 18-23. Claims 17-20 are new. Support for claims 17 and 20 can be found within claims 1-3. Support for claims 18-19 can be found on page 15 lines 11-20. Thus no new matter has been added.

Based upon the above considerations, entry of the present amendment is respectfully requested.

Specification

Applicant has amended the specification, as described on pages 2-7 of the present Amendment, in order to clarify the invention. Essentially, the phrases "glucagon C-terminal side 19-29 amino acid peptide region," "glucagon C-terminal side 19-29 amino acid peptide" and "glucagon C-terminal side 19-29" were replaced in the specification with "a peptide that has the

amino acid sequence shown in SEQ ID NO: 1." Support for this amendment can be found in the present specification on page 5, lines 18-22 which reads:

> The "glucagon C-terminal side 19-29 amino acid peptide" which is expressed by the vector according to the present invention in the form of a fusion protein, means the peptide consisting essentially of totally 11 amino acids located from the 19th to 29th amino acid counted from the C-terminal of glucagon. That is, the peptide has the amino acid sequence shown in SEQ ID NO: 1 in SEQUENCE LISTING.

Thus, no new matter has been added to the specification. Applicant respectfully requests reconsideration and that the amendments to the specification be entered.

Claim Objections

The Examiner asserts that claims 5-6 were objected for improper multiple dependent claim form. Applicant respectfully traverses.

Applicant brings to the Examiner's attention the Preliminary Amendment dated July 7, 2007, wherein the improper multiple dependencies from claim 5 were removed.

Applicant respectfully requests reconsideration, withdrawal of the objection and subsequent examination of claims 5 and 6 on their merits.

Rejection Under 35 U.S.C §112, Second Paragraph, Indefiniteness

Claims 1-4 stand rejected under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter, which Applicant regards as his invention. (*See* Office Action, dated May 1, 2007 at page 10; hereinafter "Office Action")

The Examiner asserts that it is unclear what was intended by "glucagon C-terminal side 19-29 amino acid peptide region." Additionally, the Examiner also asserts that claims 1-4 recite "the body" without antecedent basis. Applicant respectfully traverses.

Applicant has amended claims 1-4, without prejudice or disclaimer, to further clarify the invention. Specifically, claim 1 is amended to recite "a peptide having the amino acid sequence shown in SEQ ID NO: 1," and removes the term "the body" so as to avoid confusion. Applicant respectfully requests reconsideration, and subsequent withdrawal of the present rejection.

Rejection Under 35 U.S.C §112, First Paragraph, Enablement

Claims 1-4 stand rejected under 35 U.S.C. §112, first paragraph as failing to comply with the enablement requirement. The claims contain subject matter not described in the specification in such a way as to enable one skilled in the art to make or use the invention.

The Examiner asserts that the claims are drawn to a vector with the intended use of "gene therapy," the claims do not limit the disease or disorder intended to be treated, and the claims do not recite any therapeutic gene comprised by the vector.

As suggested by the Examiner, Applicant has amended claims 1-4, without prejudice or disclaimer, to remove "gene therapy" from the claims, thus obviating the Examiner's basis for the rejection.

Applicant respectfully requests reconsideration, and subsequent withdrawal of the present rejection.

Rejection Under 35 U.S.C §103(a), Obviousness

Claims 1, 3 and 4 stand rejected under 35 U.S.C. §103(a) as unpatentable over Kim *et al.*, *Biotech. Bioeng., 69(4): 418-428, (2000)* (hereinafter, "Kim *et al.*") in view of Conradt *et al.*, *J. Biol. Chem. 264(29): 17368-17373, (1989)* (hereinafter, "Conradt *et al.*").

Claims 1-4 stand rejected under 35 U.S.C. §103(a) as unpatentable over Kim *et al.*, in view of Conrad *et al.*, and in view of Saunders *et al.*, U.S. Patent No. 5,486,599 (hereinafter, "Saunders *et al.*").

Claims 1-4 stand rejected under 35 U.S.C. §103(a) as unpatentable over Kim *et al.*, in view of Dorai *et al.*, BIO/TECHNOLOGY 12: 890-897, (1994) (hereinafter, "Dorai *et al.*").

Reconsideration and withdrawal of the above rejections is respectfully requested based on the following considerations.

The Examiner asserts that Kim *et al.* teach that glucagon, including residues 19-29 can serve as a binding partner for affinity chromatographic purification of recombinantly expressed fusion proteins. The Examiner also asserts that the Examples within Kim *et al.* include a prokaryotic expression vector encoding a fusion of glucagon to the N-terminus of IL-2 and purification of the expressed fusion protein on an affinity column comprising a glucagon receptor. The protein of interest can then be separated from the glucagon purification tag by enterokinase cleavage. Applicant respectfully traverses.

The Examiner mischaracterizes the disclosure of the Kim *et al.* reference as well as the combination of Kim *et al.* to that of the other cited references.

As the Examiner indicated in the Office Action, page 7, Kim *et al.* do not disclose or suggest a mammalian expression vector. Kim *et al.* teach a purification technique based on the

specific molecular interactions of the α-helical peptide, human glucagon. Although the particular molecular interactions of glucagon provided a rationale for using the peptide as the fusion expression partner to achieve high productivity of foreign proteins both *in vivo* (in bacterial fusion-expression system) and *in vitro* (in affinity column chromatography), the method indicates that the fusion of glucagon peptide(s) effectively promoted homogeneous aggregate formation of recombinant proteins while avoiding intermolecular crosslinking by disulfide bridges (*See* Abstract, Kim *et al.*). In other words, the method of creating the fusion protein (which includes glucagon) by Kim *et al.* was the production of a better inclusion body (aggregate) that is cleaner within *E. coli.* This improved inclusion body provides a better purification step for a bacterially produced protein.

Two domains of glucagon are discussed in which these domains determine specificity of molecular interaction and aggregate size of recombinant proteins (*Id.*) An N-terminal domain of glucagon molecule (Phe6-Tyr10-Tyr13) could be a certain hydrophobic moiety involved in intermolecular self-association (probably, via helix-helix docking), while a C-terminal domain (Phe22-Trp25-Leu26) seems to critically affect the oligomer size in the off-pathway aggregation of synthesized fusion proteins (*Id.* emphasis added). An N-terminal extracellular domain of human glucagon receptor was recombinantly expressed in *Escherichia coli,* immobilized to a chromatography column, and efficiently **renatured** to a conformation that attains high specificity in interaction with N-terminus glucagon molecules of recombinant fusion proteins. (*Id.* emphasis added).

In heterologous expression of many pharmaceutically important proteins, *in vivo* accumulation of polypeptide chains in the form of aggregated non-native states is a potential fate

in **bacterial** cytoplasm (See Kim *et al.*, Introduction, citing Hendrick and Hartl, 1993; Wetzel, 1994; emphasis added). Because isolation of the inclusion bodies is the most efficient initial step in the purification process, **followed by refolding *in vitro*** (Cleland, 1993), *in vivo* formation of homogeneous aggregates (which favors efficient conversion of non-native aggregates to native state) by a specific intermolecular interaction is of great advantage compared to covalently crosslinked heterogeneous multimers. (*Id.* emphasis added).

The inclusion body problem is one of intracellular polypeptide folding and association. During the *in vitro* refolding of certain proteins, aggregates often form from partially folded intermediates in an off-pathway step, and the properties of inclusion bodies are consistent with a similar origin: off-pathway steps from intermediates in intracellular chain folding and association pathways (*Id.*, citing Hasse-Pettingell and King, 1988; emphasis added). Within cells, nascent chains are forming sequentially on the ribosome, and must reach their native state without the intervention of a major change of heterologous environment. The ribosome, factors interacting with the nascent chains, cofactors and prosthetic groups, cytoplasmic membranes, chaperones, and the intracellular cytoplasmic environment including temperature and ionic composition, **are all factors that can influence the outcome of *in vivo* folding processes** (*Id.*, citing Mitraki and King, 1989; emphasis added).

A proper obviousness inquiry requires consideration of three factors: (1) the prior art reference (or references when combined) must teach or suggest all the claim limitations; (2) whether or not the prior art would have taught, motivated, or suggested to those of ordinary skill in the art that they should make the claimed invention (or practice the invention in case of a claimed method or process); and (3) whether the prior art establishes that in making the claimed

invention (or practicing the invention in case of a claimed method or process), there would have been a reasonable expectation of success. *See* M.P.E.P. § 2143.

Graham v. John Deere, 383 U.S. 1, 17, 148 USPQ 459, 467 (1966), has provided the controlling framework for an obviousness analysis. A proper analysis under § 103(a) requires consideration of the four *Graham* factors of: determining the scope and content of the prior art; ascertaining the differences between the prior art and the claims that are at issue; resolving the level of ordinary skill in the pertinent art; and evaluating any evidence of secondary considerations (e.g., commercial success; unexpected results). 383 U.S. at 17, 148 USPQ at 467.

The teaching, suggestion, motivation test is a valid test for obviousness, but one which cannot be too rigidly applied. *See KSR International Co. v Teleflex Inc.*, 82 USPQ2d 1385, 1395 (U.S. 2007). While the courts have adopted a more flexible teaching/suggestion/motivation (TSM) test in connection with the obviousness standard based on the *KSR v. Teleflex* case which involved a mechanical device in a relatively predictable technological area, it remains true that, despite this altered standard, the courts recognize inventors face additional barriers in relatively unpredictable technological areas as noted in *Takeda Chemical Industries, Ltd. v. Alphapharm Pty., Ltd.*, 83 USPQ2d 1169 (Fed. Cir. 2007) (since TSM test can provide helpful insight if it is not applied as rigid and mandatory formula, and since, in cases involving new chemical compounds, it remains necessary to identify some reason that would have led chemist to modify known compound, in particular manner, in order to establish *prima facie* obviousness of new compound).

Kim *et al.* teaches the use of a purification system in which aggregates, multimeric forms of a fusion protein (that are not in their native 3-dimensional conformation) are produced more

efficiently by the use of a full length glucagon (or mutant variants thereof) in bacteria. The proteins that are produced through the Kim *et al.* process are formed within *E. coli*. When proteins are over expressed, the bacteria shuttles these proteins into what is known as inclusion bodies. Inclusion bodies are misfolded polypeptides which are stored until they could be further processed (*i.e.*, refolded to the native conformation of the protein) or broken down by proteases.

One skilled in the art would recognize Kim *et al.* as a process for bacterially expressed proteins in which high purity inclusion bodies are sought. The skilled artisan would also recognize that the process has many pitfalls; mainly once the fusion protein is made as an inclusion body, the mis-folded polypeptides that constitute the inclusion bodies must be refolded into its 3-dimensional "native" conformation in order for the protein to be biologically "active." The refold process is not a trivial event; a refold program has to deal with many factors (buffer conditions, pH, chaotropic agents, reducing agent, refolding times, temperature, etc.) and has to be weighed against its potential for producing correctly folded protein (which, depending on the amount of disulfides present in the protein, can make the potential slight).

The skilled artisan would also recognize that the Kim *et al.* reference would not delve into the issues of correct glycosylation of a protein, since *E. coli* does not have the physical mechanisms to produce glycosylated protein as does a mammalian-based cell line. A skilled artisan working with a mammalian cell line would first direct his/her efforts toward a process (purification, expression) for mammalian cells.

As in *Takeda Chemical Industries, Ltd. v. Alphapharm Pty.*, cited above, it remains necessary to identify some reason that would have led a biochemist to modify Kim *et al.* in a particular manner, in order to establish a *prima facie* case of obviousness. As a practical matter,

Kim *et al.* teaches away from the present invention since a skilled artisan, such as a biochemist, would not use the process or methodologies in Kim *et al.* since the ultimate goal in Kim *et al.* is to promote an unglycosylated fusion protein within an inclusion body in bacteria. The inclusion body would be of high purity, but the artisan would then have to proceed further and process the inclusion bodies and then correctly fold the fusion protein. In contrast, an artisan who produces a fusion protein within mammalian cells would not be able to use the approach in Kim *et al.* since a purification scheme would involve cells in which the fusion protein is already properly folded and glycosylated (if the protein is amenable to glycosylation). The purification scheme would be different since the target protein as well as contaminants would be properly folded. The proteins will behave differently when properly folded and glycosylated (binding affinities to columns, as well as protein-protein interactions), so the artisan will have to use a vastly different regimen than that of Kim *et al.* Therefore, Kim *et al.* teaches away from the present invention and the *prima facie* case of obviousness has not been met.

If the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. In *re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959) (The court reversed the rejection holding the "suggested combination of references would require a substantial reconstruction and redesign of the elements shown in [the primary reference] as well as a change in the basic principle under which the [primary reference] construction was designed to operate." 270 F.2d at 813, 123 USPQ at 352.).

The combination of Kim *et al.* to that of the other cited references is inapplicable since the primary goal of the "one step purification" of Kim *et al.* (a bacterial expression system)

proposed by the Examiner is not combinable with the cited references that utilize a mammalian expression system. The Examiner within the Office Action on pages 7-8 asserts that it would have been obvious to one of ordinary in skill in the art at the time of the invention to modify the purification method of Conradt *et al.*, by expressing IL-2 as a fusion to glucagon, as taught by Kim *et al.*, since the method of Kim *et al.* allows a single step purification of the fusion protein. Again the Applicant respectfully traverses.

By the above, the Examiner proposes that a skilled artisan would make a fusion protein using glucagon as a single step purification procedure that produces a fusion protein within an inclusion body of *E. coli* would be obvious to a skilled artisan who is trying to make a properly folded protein within a mammalian expression system. This is not logical for the artisan since the mere fact that Kim *et al.* produce unfolded protein that is not glycosylated. The Kim *et al.* step would not be a step that would enhance the purification scheme as proposed by the Examiner, but would be a drawback and would not be considered. The approach in combining Kim *et al.* with the other cited references does not make sense and appears to follow within the lines of *re Ratti*, discussed above.

Alternatively, in the above cited rejections, Kim *et al.* is the primary reference in which it discloses a fusion protein including the full length of glucagon. However, Kim *et al.* are silent about the fusion of the peptide whose amino acid sequence is shown in SEQ ID NO: 1.

In contrast, as clarified by the above-described Proposed Amendments, the glucagon moiety of the fusion protein encoded by the vector of the present invention contains the part of the glucagon, the amino acid sequence of the part of the glucagon being shown in SEQ ID NO: 1. That is, the partial glucagon fused in the protein encoded by the vector of the 19th-29th amino

acid of glucagon (counted from the N-terminus) and does not contain a glucagon region other than the 19th-29th amino acid region (the peptide whose amino acid sequence is shown in SEQ ID NO: 1 is hereinafter referred to as "glucagon 19-29" for short).

As expressly stated in the specification on page 3, first complete paragraph, this feature brings about unexpected superior effects. That is, glucagon 19-29 itself does not have any physiological activity. In addition, since glucagon 19-29 is well conserved in various mammals, it does not substantially induce an immunological reaction while it can be quantified by immunoassay with high sensitivity using a commercially available immunoassay kit.

Therefore, glucagon 19-29 is an ideal label for the desired protein to be produced in the body in gene therapy.

Kim *et al.* are totally silent about the inclusion of the glucagon 19-29 (i.e., not full length) as well as the above-mentioned unexpected superior effects brought about thereby. In Kim *et al.*, since the entire glucagon is fused, the glucagon moiety has a physiological activity.

In addition, all of other secondary references above are silent about a fusion protein containing a glucagon moiety. Therefore, even if any one or more of above cited references are combined with Kim *et al.*, the present invention cannot be reached.

In conclusion, the 35 USC §103(a) rejections on obviousness against the cited combined references does not meet the prima facie standard of obviousness and therefore were improper. Applicant respectfully requests reconsideration and subsequent withdrawal of the above rejections.

In view of the above amendment, applicant believes the pending application is in condition for allowance.

CONCLUSION

In view of the above remarks, it is believed that claims are allowable.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Paul D. Pyla, Reg. No. 59, 228, at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.14; particularly, extension of time fees.

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Respectfully submitted,

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